

Appln No.: 09/938,901 Applicant(s): Kuramitsu, et al.
NOVEL DNA REPAIR ENZYMES, NUCLEIC ACIDS
ENCODING DNA REPAIR ENZYMES AND METHODS OF **USING THEM** 

FIG. 1



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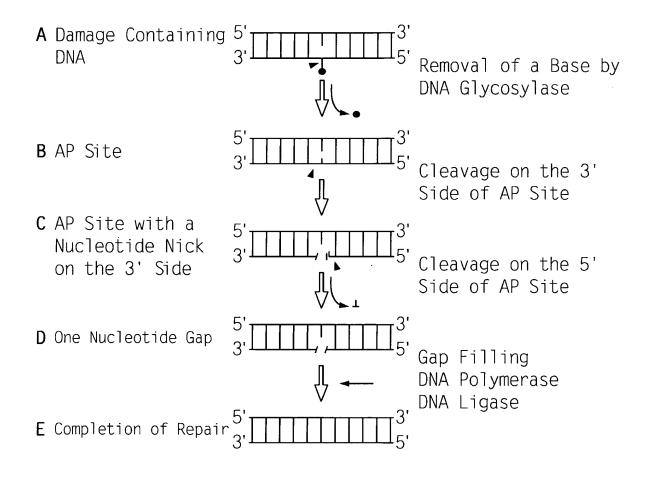


FIG. 2

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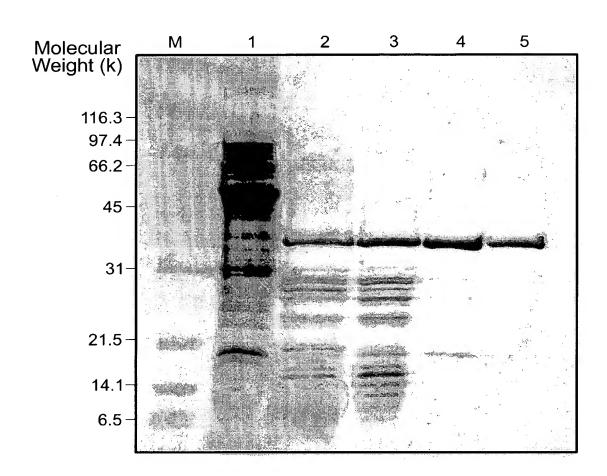


FIG. 3



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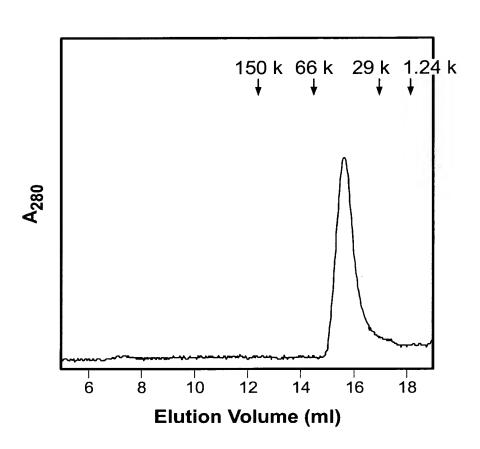


FIG. 4



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1 51 CDGLARQPEEVVLQASVSSYHLFRDVAEVTAFRGSLLSMYDGE-KRDLPWRRAEDEMDLDRRAYAWWYSEWLDQTDWATVINNYIRGMM 139 51 CDGLARQPEEVVLQASVSSYHLFRDVAEVTAFRGSLLSMYDGE-KRDLPWRRAEDEMDLDRRAYAWWYSEWLDQTDWATVINNYIRGMM 139 51 CDGLARQPEEVVLQASVSSYHLFRDVAEVRFRESLIDFYDKT-KRILPWRKKECIPPSEDSPLEDWEQPVQRLMEVLNSFIMLQQTDWATVINNYIRGMM 88 52 MQASQFSAQVLDMYDKYGRKTLPWQIDKTPMYWWLSEVMLQQTDVATVIIPMYFERFM 56 53 MAKAKRLEILIRLREN-NPHPTTELNFSSPFELLIAVLLSAQATDVSVNKATAKLY 55	54 ERFPIT KALAMASIE-EVLRWWOGAGYYR-RAEHLHRLARSVEELPPSFAELR-GPGLGPYTAAAWASIAFGERVAAVDGNVRYLSRLFARES 145 140 QKWPTI QOLASASIE-EVNQLWAGLGYYS-RGRRLQEGARKVVEELGGHMPRITAETLQQLLPGVGRYTAGALASIAFGQATGVVDGNVARVLCRVRALGA 237 89 ETLPTI KSCAEMENYTQVMPLWSGMGFYT-RCKRLHQACQHLAKLHPSEIPRITGDEWAKGIPGVGPYTAGAVLSIAWKQPTGIVDGNVIRVLSRALMIHS 187 57 ARFPTVTOLANAPLD-EVLHLWTGLGYYA-RARNLHKAAQQVATLHGGKFPEIJFEENA-ALPGVGRSTAGATLGSLSKHFPILDGNVKRVLARCVAVSG 153 56 PVANTPAAMLELGWE-GVKTYIKTIGLYNSKAENIJIKTCRILLEQHNGEVPEDRAALE-ALPGWGRKTANNVLNTAFGWPTIAVDITHIFRVCNRTQFAPG 153	146 -PKEKELFM AQGL PEGVDPGVWNQALMELGATVCLPKRPRCGACPLGAFCRG	211 RRAK	267 GEVRHALTHRRLRVEWR-GALWEGEGEDPWKRPLPKLMEKNLRKM-P
Tth Muty Hsa Muty Spo Muty Eco Muty Eco EndoIII	Tth Muty Hsa Muty Spo Muty Eco Muty Eco EndoIII	Tth Muty Hsa Muty Spo Muty Eco Muty Eco Endolll	Tth Muty Hsa Muty Spo Muty Eco Muty Eco Endolll	Tth Muty Hsa Muty Spo Muty Eco Muty

Tth (Thermus thermophilus HBB), Hsa (Homo sapiens), Spo (Schizosaccharomyces pombe), Eco (Escherichia coli)
# Residue essential for N-glycosylase activity \* Residues constituting an iron-sulfur cluster



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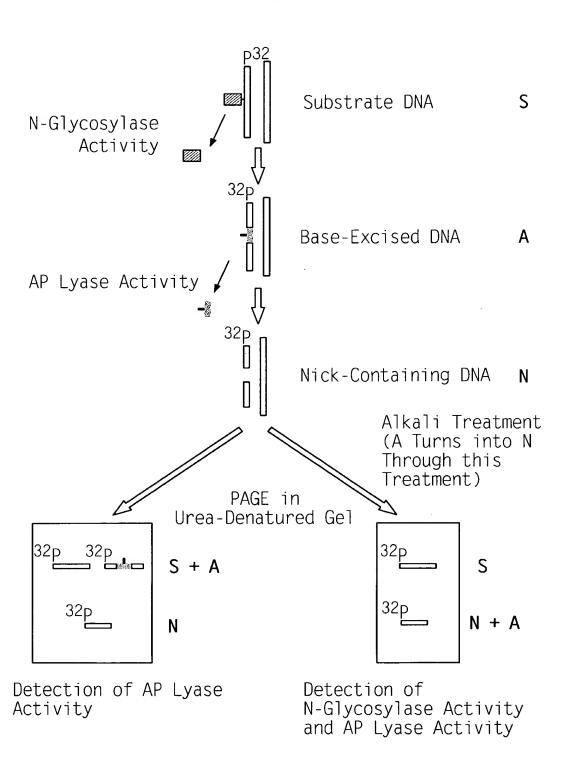
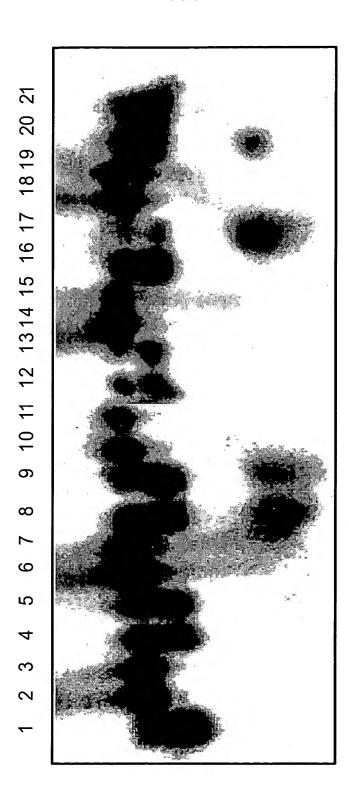


FIG. 6



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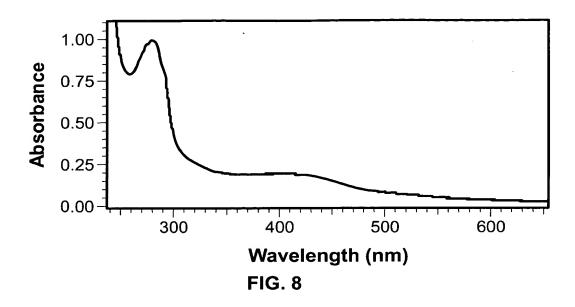


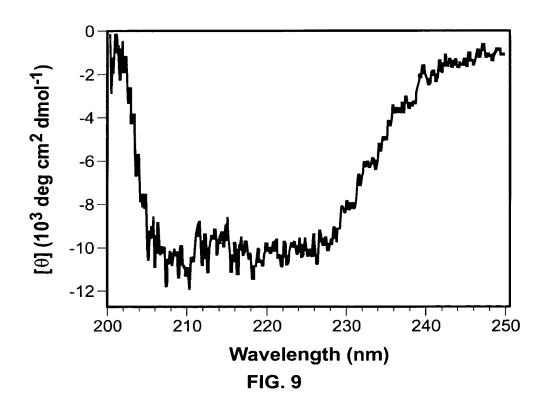


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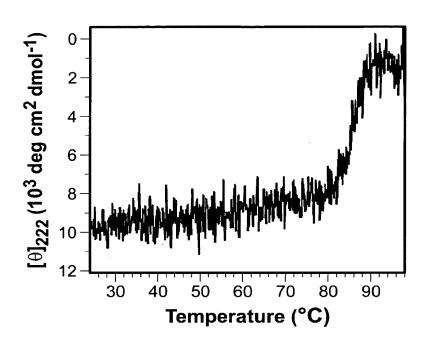


FIG. 10



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 $5'-[^{32}P]$ AGATCTTGACGGGGAAAYCCGAATTCGGCGAACGTGGCGAG-3'3'-AATCTAGAACTGCCCCTTTXGGCTTAAGCCGCTTGCACCGCTCTT-5'

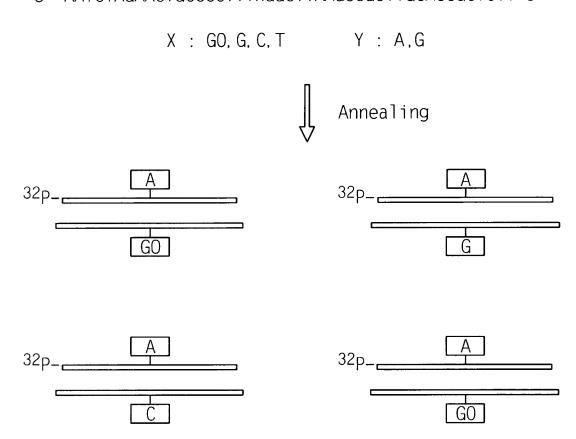
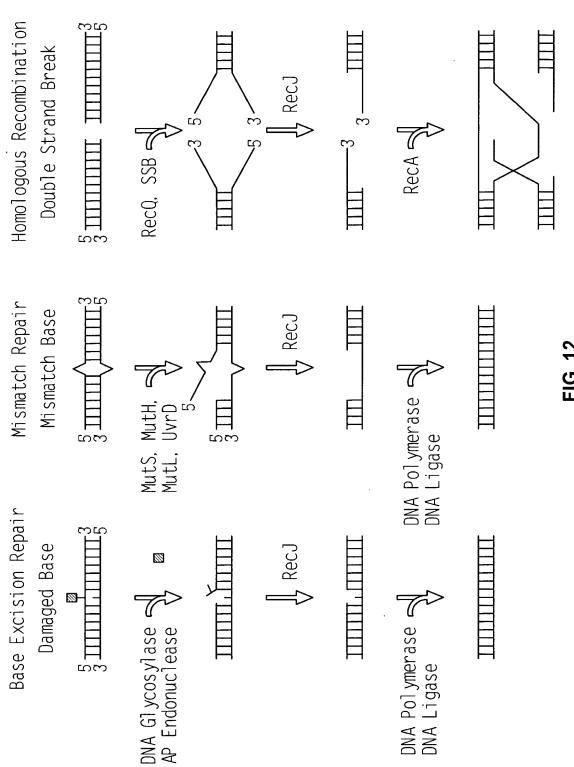


FIG. 11



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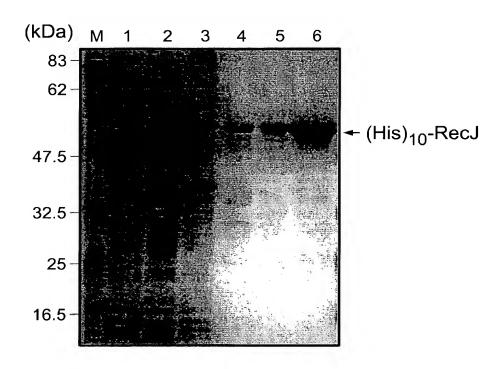


FIG. 13

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RecJ_Tt RecJ_Ec RecJ_Aa RecJ_Hp RecJ_Hi	[73] [78] [47]	Motif I KRIRVHGDYDADGLTGTAILV TRIIVVGDFDADGATSTALSV KRIIIYGDYDVDGITGTAILY TEILVVGDYDADGVISSAIMA QKIVIVGDFDADGATSTALSV	LAMRSLG RVLKLLG AKFFESLN	[100] [100] [105] [74] [95]		
PPX1_Sc PRUNE_Dm		TICVGNESADMOSTASATTYS HLVMGNESCOLOSAVSAVTLA	1 1	[57] [60]		
		Motif II			Motif III	
RecJ_Tt RecJ_Ec RecJ_Aa RecJ_Hp RecJ_Hi	[129] [131] [133] [102] [126]	SDLFLTVDCGITNHAELRE AQLIVTVDNGISSHAGVEH GDFLITVDNGTSAVEEIDQ APLIITVDNGINAFEAARF VQLLMTVDNGVSSFDGVAF	[147] [49] [151] [120] [144]	[153] [155] [154] [126] [150]	VEVIVTPHHTPGK [165] IPVIVTPHHLPGD [165] LETVVIPHHNVPP [166] YTLIITPHHCLHH [138] IRVLVTPHHLPPE [162]	
PPX1_Sc PRUNE_Dm	[120] [88]	ELNS <mark>YLVD</mark> NNDTPKNLKNY PLVC <u>EMWD</u> CRARVALPRRY	[138] [106]	[141] [129]	NVVGIIDHHFDLQ [153] NVTEILDHRPLED [141]	
		Motif IV			Specific Motif	
RecJ_Tt RecJ_Ec RecJ_Aa RecJ_Hp RecJ_Hi	[210] [226] [215] [189] [219]	YADLAAVGTIADVAPLWGW LLDLVALGTVADVVPLDAN FLDLVALGLLADYMPVNPV LLCLAGVATIADMMPLTFF LLDLVALGTIADVVPLDQN	[228] [244] [233] [207] [237]	[386] [422] [404] [372] [415]	DLLLRYGGHKEAAGFAM [402 GMMLKFGGHAMAAGLSL [438 DMFLKWGGHDKAMGLTL [420 SLLLGYGGHRQACGLSV [388 NMILKFGGHAMAAGLSI [431	3] 3] 3]
PPX1_Sc PRUNE_Dm	[191] [183]	IALLIMGATLIDTSNMRRK VAQLLHATIVLDTINFAPA	[209] [201]			

Tt : Thermus thermophilus HB8. Ec : Escherichia coli, Aa : Aquifex aeolicus,

Hp : Helicobacter pyroli, Hi : Haemophilus influenzae Rd,  $\ensuremath{\mathsf{Sc}}$  : Saccharomyces cerevisiae,  $\ensuremath{\mathsf{Dm}}$  :  $\ensuremath{\mathsf{Drosophila}}$  melanogaster

FIG. 14



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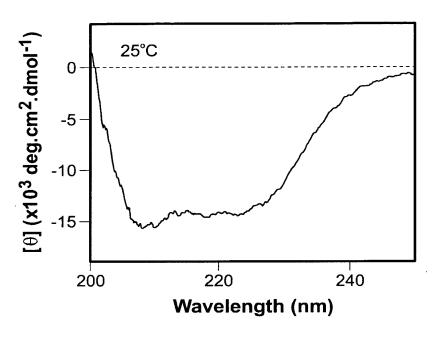


FIG. 15

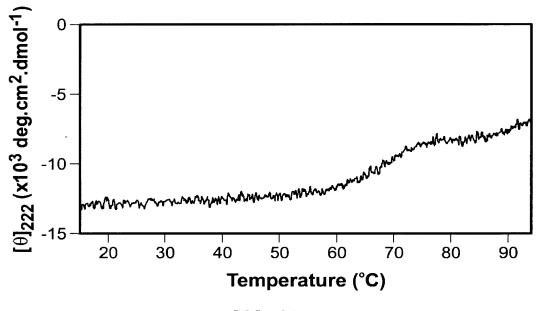


FIG. 16



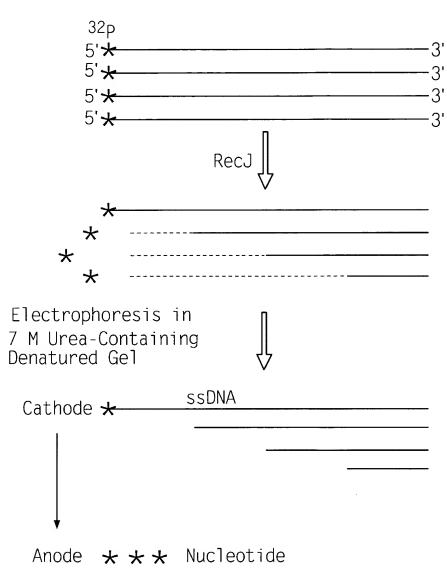


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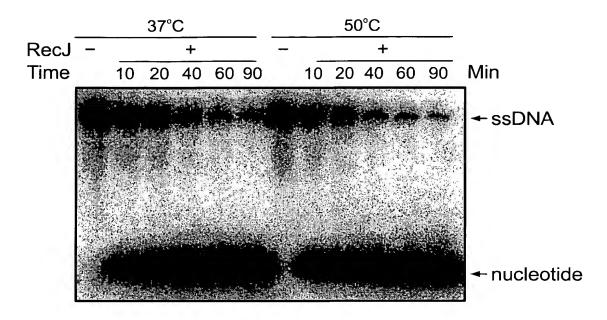
Substrate DNA:49'merssDNA

5' -ACTACTTGGTACACTGACGCGAGCACGCAGGAGCTCATTCCAGTGCGCA-3'

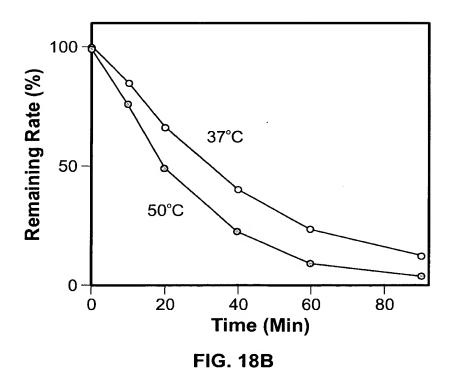


**FIG.17** 

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**FIG. 18A** 





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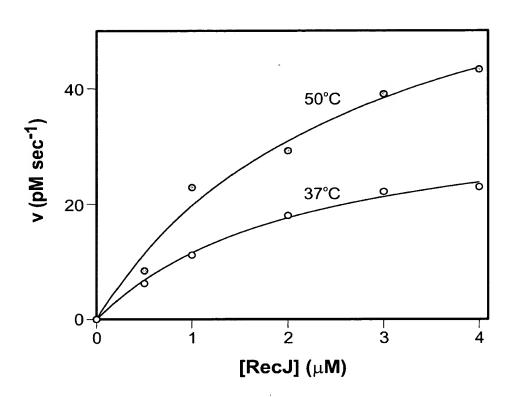


FIG. 19



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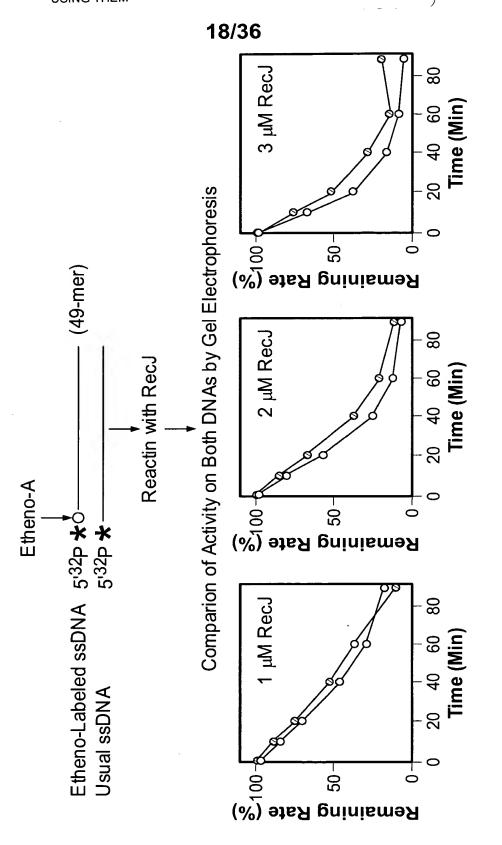


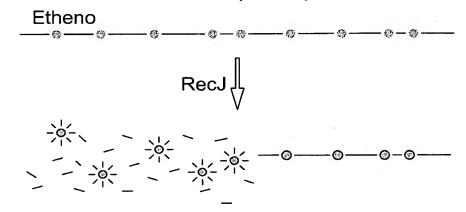
FIG. 20



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# Substrate DNA : Etheno-Labeled Bovine Thymus ssDNA (e DNA)



#### Fluorescence Spectrum

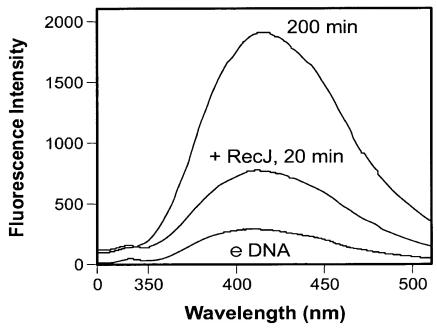
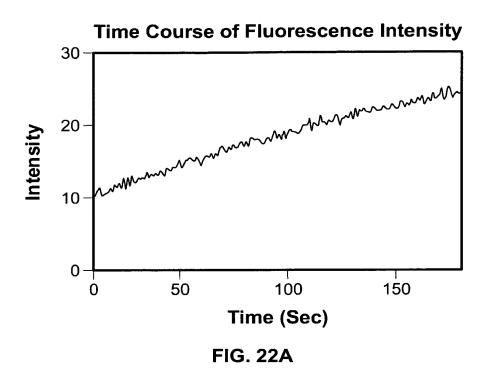


FIG. 21



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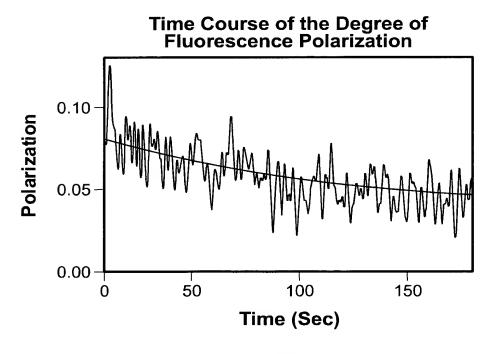


FIG. 22B

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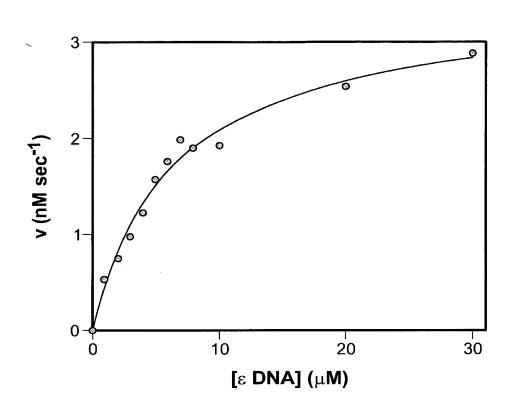


FIG. 23

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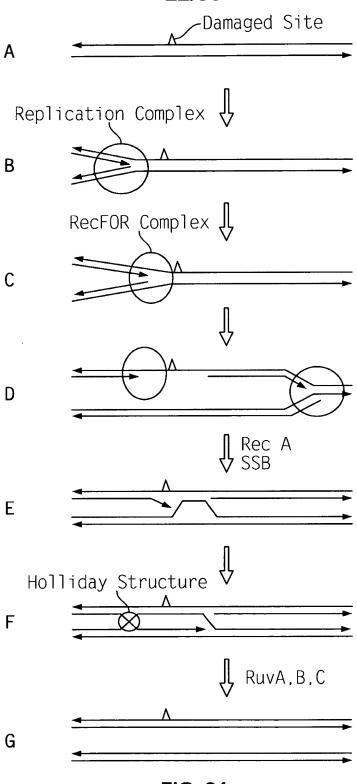


FIG. 24



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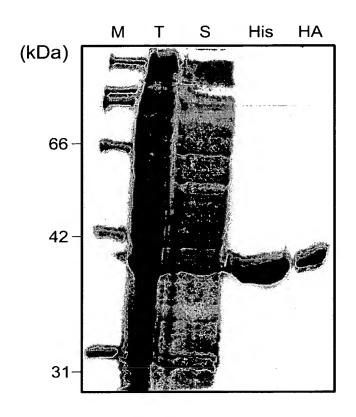


FIG. 25



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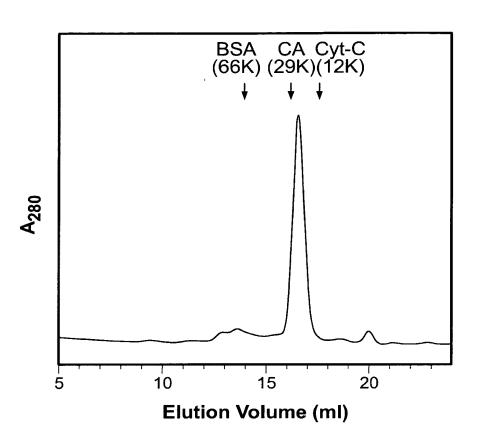


FIG. 26



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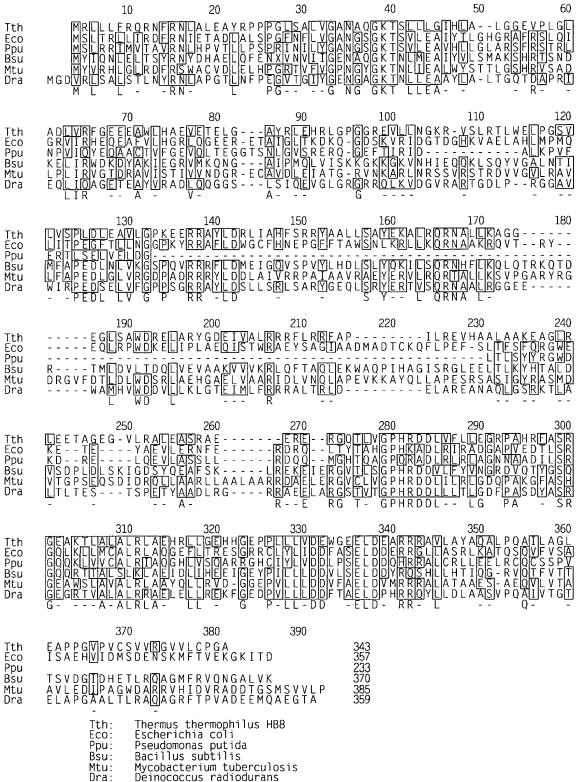


FIG. 27

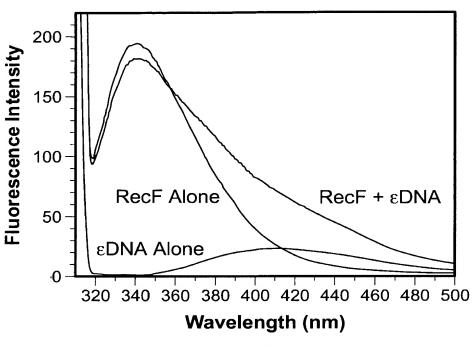
Appln No.: 09/938,901 ENCODING DNA REPAIR ENZYMES AND METHODS OF

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**FIG. 28A** 

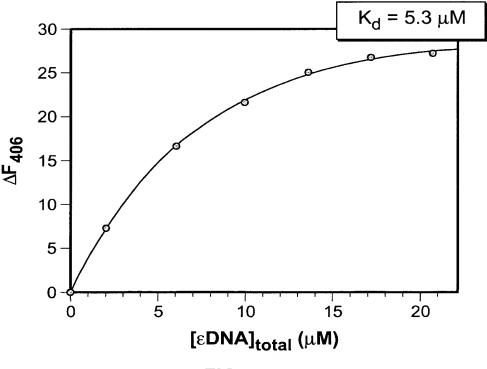


FIG. 28B

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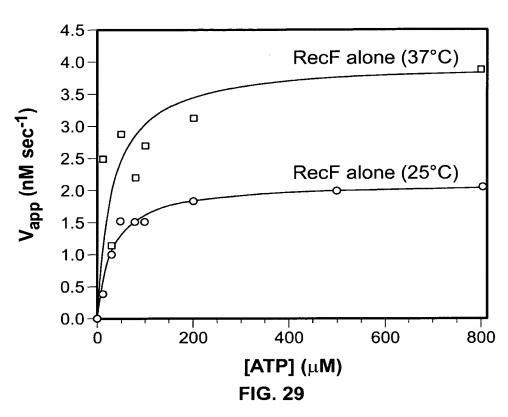
Applicant(s): Kuramitsu, et al.

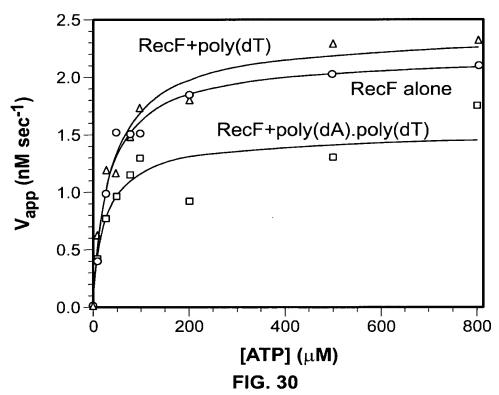
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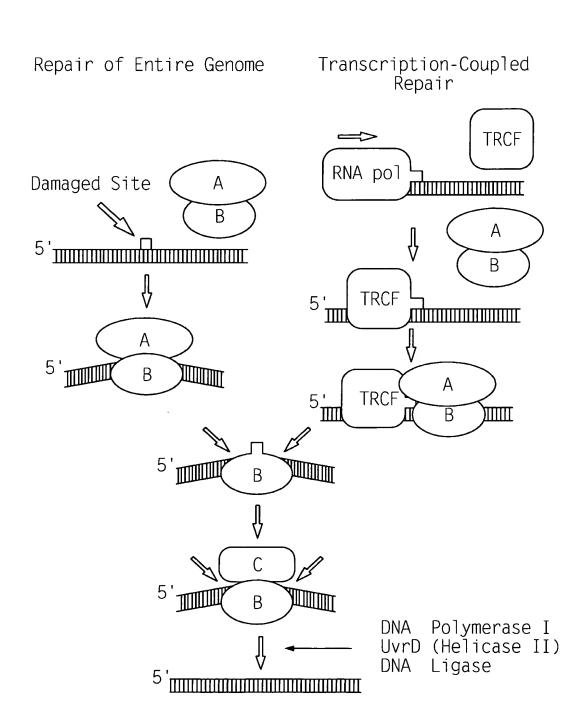


FIG. 31



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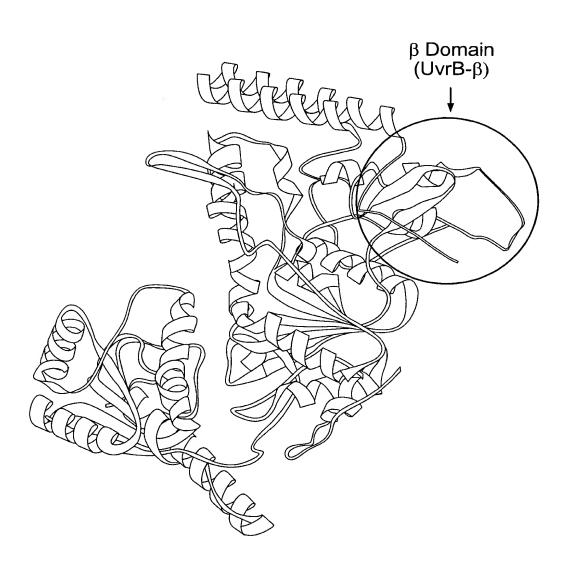


FIG. 32



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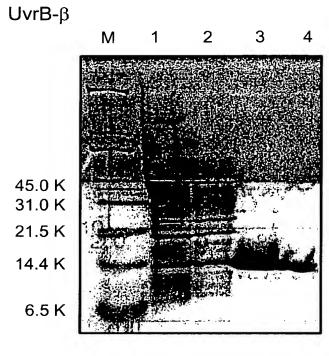


FIG. 33A

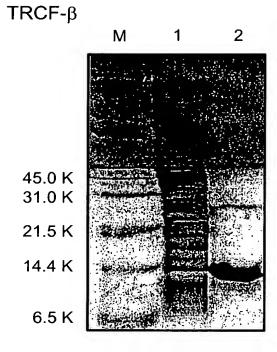


FIG. 33B



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184 UvrB-B TRCF-B 86 WRLL 113 UvrB-B 215 185 DLSPGRFRAKGEV 148 TRCF-B 114 UvrB-B 216 GDEWERISQVHPVTG-ERLRELPG 236 163 TRCF-B 149 \*\*\* \*\* 242 \*Identical Amino Acid Residues UvrB-B 237 ---FW 172 . Homologous Amino Acid Residues TRCF-B 164 SKKV

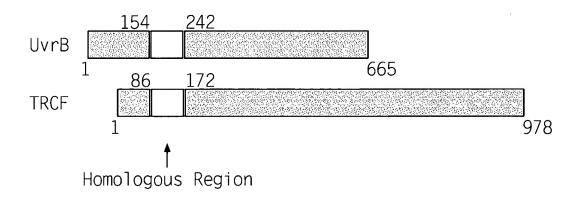


FIG. 34



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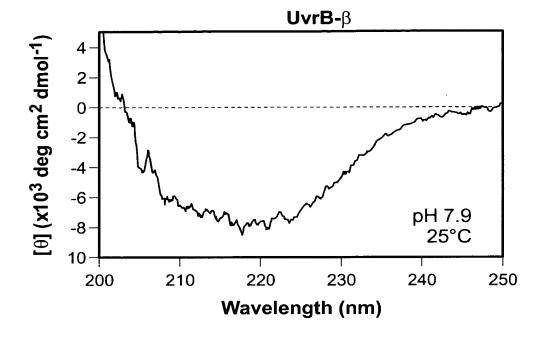


FIG. 35A

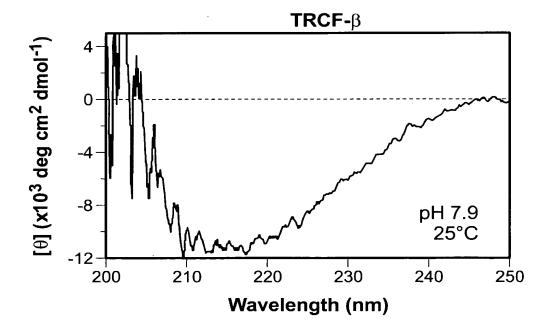


FIG. 35B

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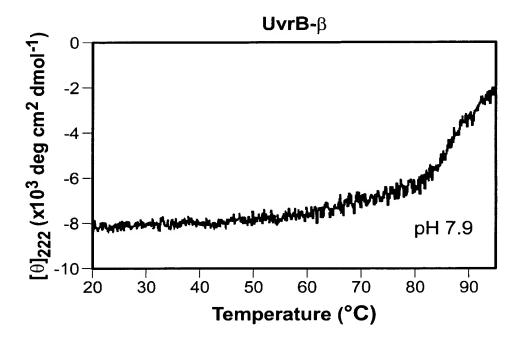


FIG. 36A

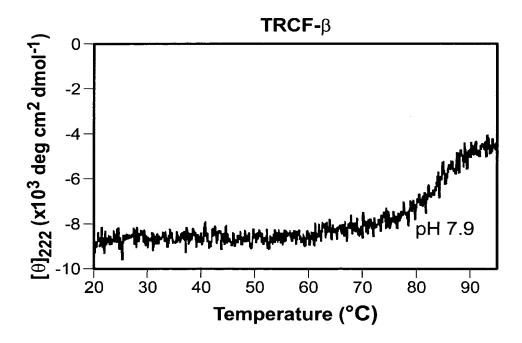
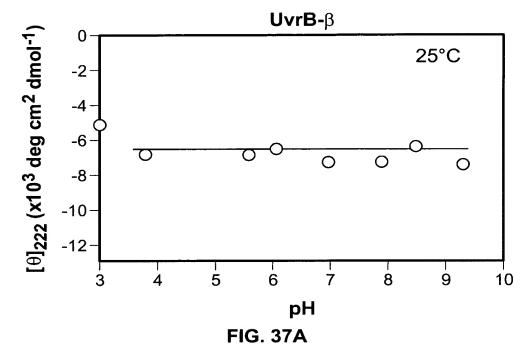


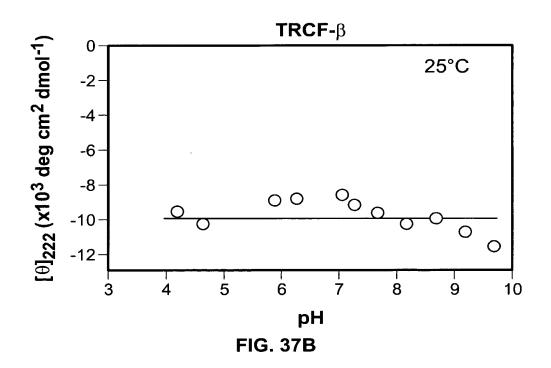
FIG. 36B



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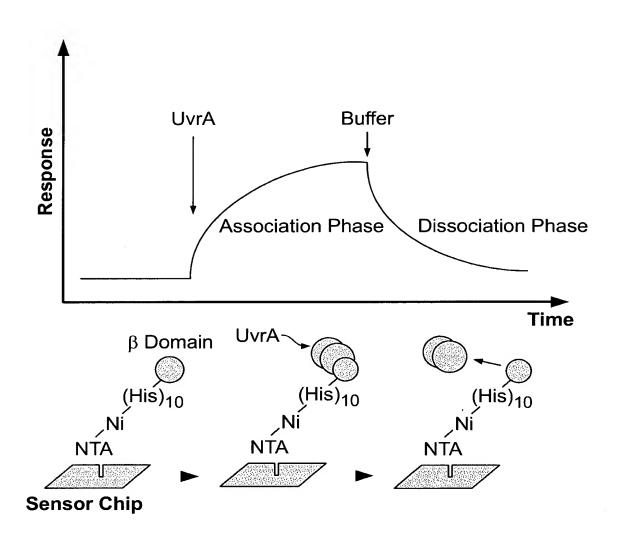


FIG. 38



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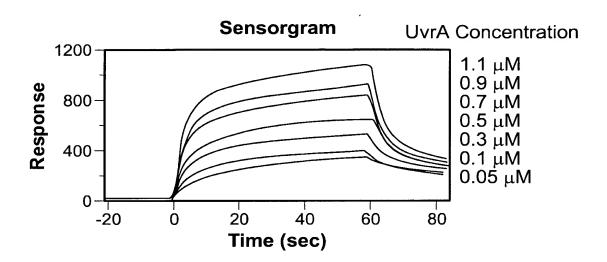
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# **Analytical Results**

	K <sub>d</sub> (x10 <sup>-6</sup> M)		$k_{on}$ (x10 <sup>5</sup> M <sup>-1</sup> S <sup>-1</sup> )		$k_{off} (x10^{-1}S^{-1})$	
	-ATP	+ATP	-ATP	+ATP	-ATP	+ATP
UvrB-b	2.6	0.4	2.0	1.5	5.2	0.6
TRCF-b	1.3	0.5	1.0	1.5	1.3	0.7

FIG. 39

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